Ms #2-35
Unpublished Surveys Control
C. F. No.
X-ref.
Card index
INTERMOUNTAIN STATION
Central Reference File
No. 3.4163-62
Coeur d'Alene, Ida. Station

UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE

Project | Date | Author

TITLE

CRITICAL LOW TEMPERATURES FOR THE BLACK HILLS BEETLE

1937-38

By

N. D. Wygant, Assistant Entomologist

Forest Insect Laboratory
Fort Collins, Colorado
June 30, 1938

SUBJECT—

INDEX NO.—

U. S. GOVERNMENT PRINTING OFFICE 8—9742

FILE COPY

MISSOULA FOREST INSECT LABORATORY

CRITICAL LOW TEMPERATURES FOR THE BLACK HILLS BEETLE

1937-38

By

N. D. Wygant, Assistant Entomologist

Forest Insect Laboratory
Fort Collins, Colorado
June 30, 1938

TABLE OF CONTENTS

	Page No.
Introduction	1
Equipment	2
Methods	3
Source of Material and Storage	5
Results of Experiments	6
Critical Temperatures in Relation to Season	6
Critical Temperatures in Relation to Host	8
Critical Temperatures in Relation to Locality	10
Relation of Mortality to Period of Exposure	11
Experiment to Determine Effect of Warm Temperatures after Exposure	12
Summary of Weather Records	12
Summary	14
Appendix	17

CRITICAL LOW TEMPERATURES FOR THE BLACK HILLS BEETLE

1937-38

INTRODUCTION

Critical low temperature studies on the Black Hills beetle were started during the fall of 1936 when a low temperature cabinet and other necessary equipment were purchased for the Fort Collins forest insect laboratory. The results of the first winter season tests were reported in a progress report entitled "Critical Low Temperatures for the Black Hills Beetle 1936-37," Fort Collins, Colo., Nov. 26, 1937. The following report is a continuation of the study with a few new phases of the problem. This report includes only the data collected during the season of 1937-38 but the results of the previous season are taken into consideration in the discussions.

The broken control system handicapped the work during the early part of the season, making it necessary to "hand control" the low temperature cabinet. A new control system was installed during the last of January which greatly facilitated the work.

The low temperature experiments for the season 1937-38 were conducted to determine the critical temperatures of the Black Hills beetle at various times of the year, the relation of the critical temperature range to its hosts of the region, and the relation of the critical

temperature range to locality. These relations have been fairly well worked out but there remain a few gaps in the data which should be filled during the next winter. The lethal temperature data have been obtained for naked larvae and now the insulating values of the three important hosts of this region should be determined to make the data applicable to field conditions.

EQUIPMENT

The experiments were carried out in an automatically controlled, well-type, low temperature cabinet designed by the Forest Insect Laboratory at Berkeley, California, and installed at Fort Collins, Colorado, during the summer of 1936. The original control system was unsatisfactory and a new recording resistance thermometer controller was installed during January 1938. The tube system containing the mercury ruptured again during the spring of 1937 due to the high pressure created in the system at room temperatures. Since this system, which was made up on special order, had proven unsatisfactory the makers refused to install another one like it. They allowed full purchase price of the old system on the resistance thermometer type.

Some of the advantages of the new controller are -- more sensitive; more accurate; a lower range, -40° to $+40^{\circ}$ as compared to -25° to $+25^{\circ}$ on the old system; calibration does not change while idle; and produces a record of the temperature in the cabinet during each test.

Since mortality does not occur with some of the larvae during periods of extreme hardiness, except below -25°, the lower range is very necessary. The lowest temperature obtainable with the compressor is -34°. Temperatures lower than this can be obtained by placing a block of "dry ice" in the cabinet. When using the dry ice a constant temperature is maintained by a heater such as an electric light bulb which is turned on by the automatic controller when the temperature becomes a fraction of a degree lower than the setting. The wattage of the electric light bulb can be varied in proportion to the amount of "dry ice" so that the heater is not turned on and off too frequently.

METHODS

The methods used for the most part in making the exposures of the larvae were those used in the tests during the winter of 1936 and 1937. The larvae were exposed in samples of 50 in petri dishes with individual cells. The petri dishes with the paraffin cells or pits were prepared by mixing melted paraffin wax with lamp black, pouring a sufficient amount of the mixture into the bottom of the petri dish to make a layer of wax about 1/8 inch thick, and after cooling, drilling 50 properly spaced holes into the wax with a 3/8 or 1/4 inch twist drill. One larva was placed in each hole making a sample of 50 larvae. The larvae were exposed for 2 hours and 15 minutes and a hasty count made of the frozen larvae when removed from the cabinet when it was possible to distinguish

between the frozen and unfrozen ones. Exposures were made at 2-1/2 degree intervals over the critical range.

Previous to about Nov. 15 and after Apr. 1 or when the larvae are fairly active the survivors of the exposure can be distinguished from the frozen or killed ones within a few hours after exposure. Between these two dates the larvae are dormant, and it is impossible at the present to distinguish the uninjured ones from the partially injured ones by merely looking at them and observing the body movements. The injured ones are not as active as the uninjured ones but the difference is frequently not a sufficient basis upon which to separate them. With more experience perhaps the two groups can be separated by inspection. There are always some which are killed outright and can be sorted from the others.

The exposed larvae were placed in 9x36 mm. vials with fresh ponderosa pine phloem and stoppered with cotton, one larva to each vial, soon after exposure. The uninjured ones start feeding on the phloem in 3 or 4 days while the injured ones do not feed and eventually die. This process of feeding the larvae after exposure requires a lot of additional work, but it is the only satisfactory method whereby the two groups can be separated. During extreme dormancy the freezing appears to be partial in some larvae and complete in others. The ones considered to be completely frozen are hard and cannot be dented with the lead of a pencil. The partially frozen ones are not solid but neither are they as soft as the unfrozen larvae. It seems that when a certain stage or degree of freezing is reached death is immediate and stages or

degrees below this point may result in injury which does not kill immediately but prevents the larvae from completing their development. What stage of freezing, if any, the larvae are able to go through and recover is yet to be determined.

Source of Material and Storage

The ponderosa pine infested with the Black Hills beetle larvae was collected from Big Elk Park on the Roosevelt National Forest on Oct. 20 and 30, 1937, at an elevation of approximately 7500 feet. The logs were cut into 40-inch sections and hauled to Redfeather Lakes, Colo.

The infested limber pine was collected in 40-inch log lengths from Pingree Park, Roosevelt National Forest, on Oct. 25, at an elevation of approximately 8500 feet, and taken to Redfeather Lakes.

The infested lodgepole pine was collected from Lincoln Park on the Medicine Bow National Forest in Wyoming, at an elevation of approximately 8500 feet, on Oct. 28, and taken to Redfeather Lakes.

Additional infested ponderosa pine was collected from southern Utah, southern Colorado, and the Black Hills of South Dakota, during Jan. and Feb., to determine whether there is a difference in winter hardiness due to locality. The source of this material is discussed more completely under the heading "Critical Temperatures in Relation to Locality."

All infested logs were stored at Redfeather Lakes, Colo., in a shelter constructed from slabs from a local sawmill. The shelter merely

protected the logs from the sun, rain, and snow, and allowing good ventilation. The logs were placed on end and the exposed end covered with hot paraffin wax to prevent drying out. The elevation at which they were stored was 8500 feet. A thermograph was operated under the shelter with the stored logs so that a record of the conditions to which they were subjected would be available.

RESULTS OF EXPERIMENTS

The tables containing the data on the results of each test are included in the appendix for convenience and to simplify the main text.

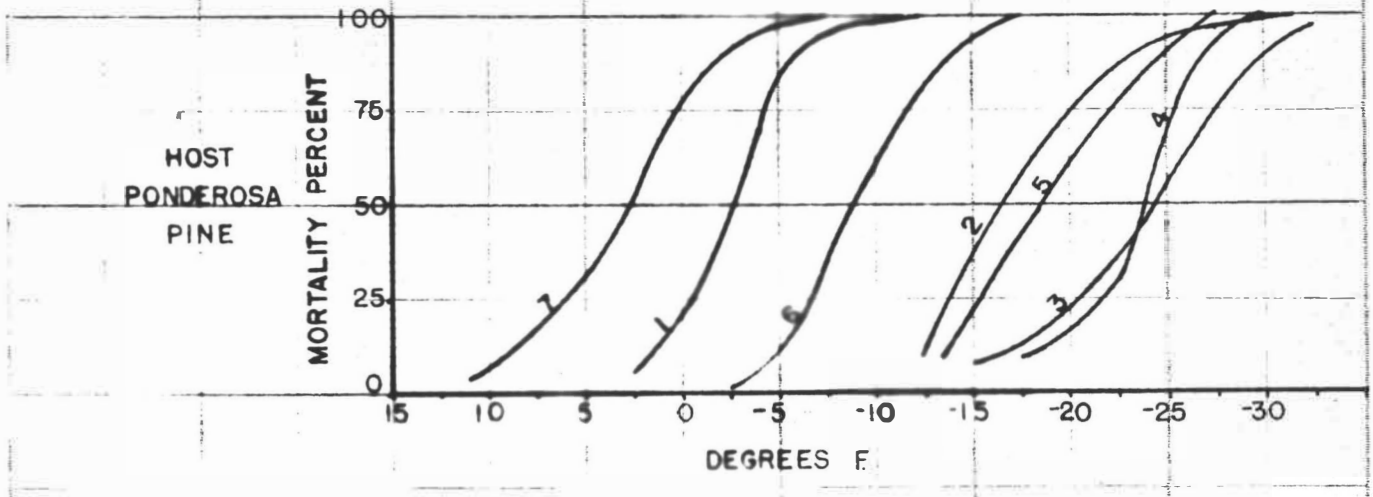
Critical Temperatures in Relation to Season

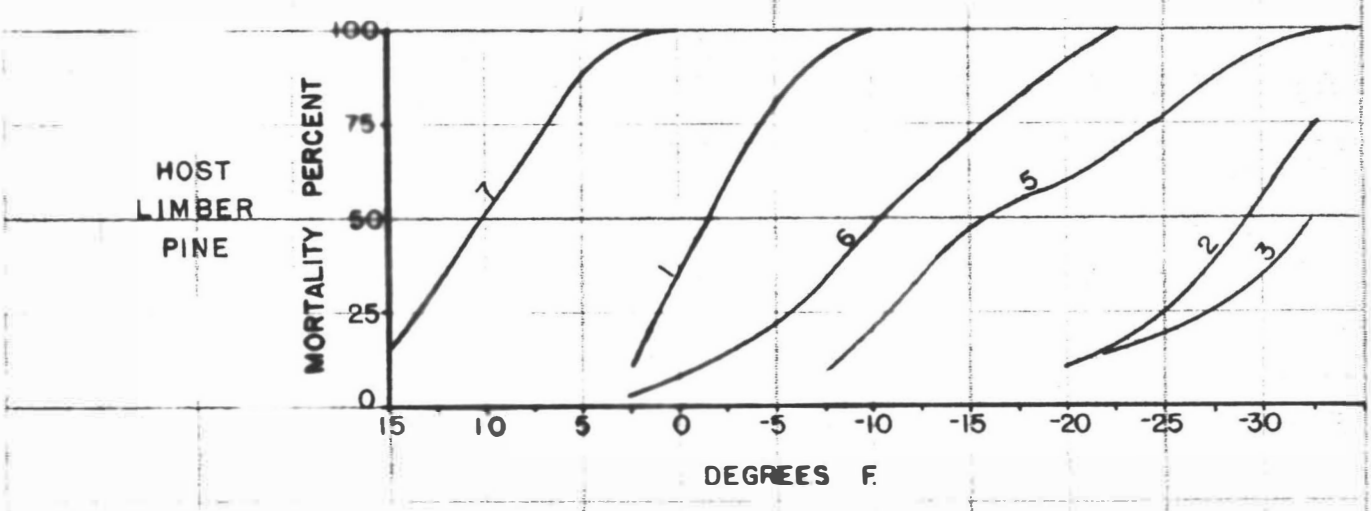
Chart I shows graphically the seasonal hardiness of the larvae of the Black Hills beetle in the three hosts -- ponderosa, limber, and lodgepole pine. The hardiness increased with the approach of winter, reaching maximum hardiness during December, January, and February. Hardiness began to decrease during the latter part of February and first of March.

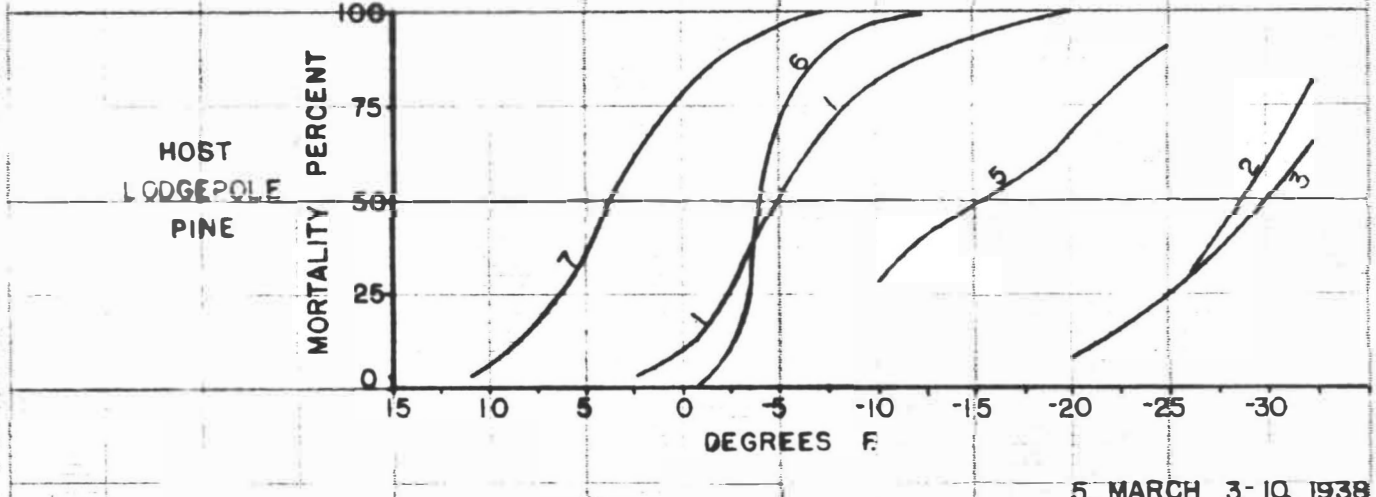
Table I gives the critical ranges for larvae at different times of the season. The hardening process was in progress when the first test was made on Nov. 4. During the period between Nov. 4 and Nov. 30 the critical range lowered 10 to 20 degrees. The test on Jan. 26-27 no doubt shows the maximum cold hardiness of the season. Mortality at that time

CHART I

CRITICAL TEMPERATURES IN RELATION TO SEASON







1. NOV. 4-6, 1937
2. NOV. 30-DEC. 1, 1937
3. JAN. 26-27, 1938
4. FEB. 9-10, 1938
5. MARCH 3-10, 1938
6. APRIL 5-7, 1938
7. MAY 11-13, 1938

did not start until temperatures of -20 to -25 degrees were reached. By March 3 when the next series of tests were made the cold resistance had decreased from 5 to 15 degrees, depending upon the host involved. The maximum temperature during the preceding week which was unusually warm was only 44° but apparently high enough to decrease the cold resistance. The cold resistance decreases gradually as spring approaches. The decrease in cold hardiness in the spring was not as rapid as the increase in hardiness in the fall. In general the periodicity of cold hardiness was about the same as that determined in the 1936-37 tests.

Table I

Summary of Critical Temperature Range in Three Hosts

Date	Critical Range of Black Hills Beetle Larvae in Three Hosts					
	Ponderosa Pine		Limber Pine		Lodgepole Pine	
	Initial	Ultimate	Initial	Ultimate	Initial	Ultimate
Nov. 4-6, 1937	2½	-12½	2½	-10	- 2½	-20
Nov. 30 - Dec. 1	-12½	-32½	-22½	68% at -32½	-22½	82% at -32½
Jan. 26-27, 1938	-20	98% at -32½*	-25	32% at -32½	-22½	60% at -32½
Mar. 3-10	-15	-27½	-10	-35	- 7½	90% at -25
Apr. 5-7	- 5	-17½	- 2½	-22½	- 2½	-12½
May 11-13, 1938	7½	- 7½	15	0	7½	- 7½

* Percent mortality at the lowest temperature obtainable with the low temperature cabinet.

Critical Temperatures in Relation to Host

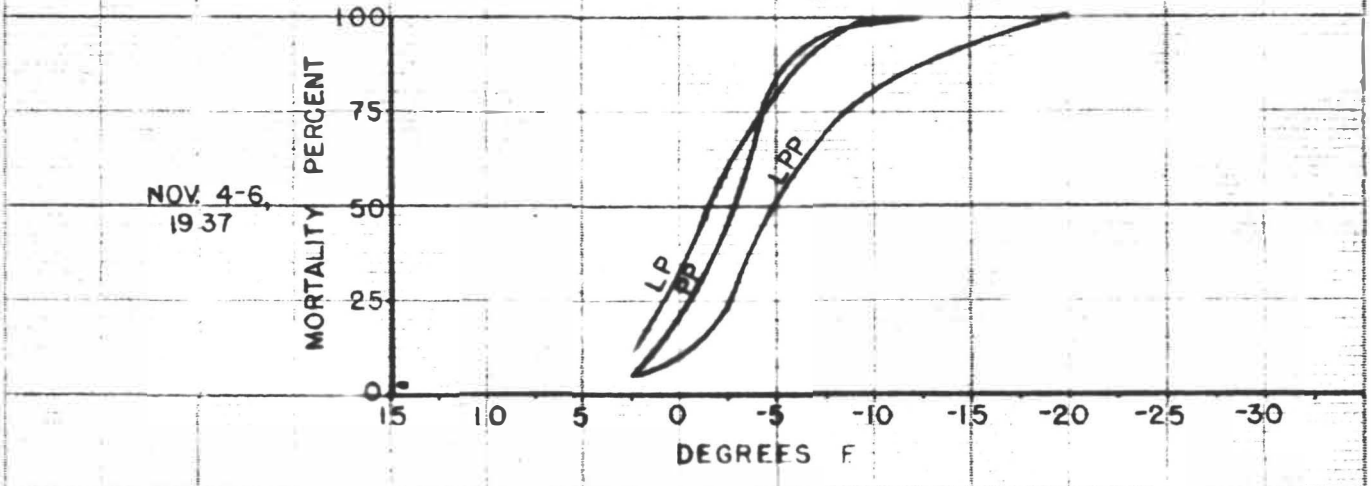
During the winter season of 1936-37 the only reliable tests made of the difference in cold resistance in three hosts were during late winter and spring. During this past season, tests were made in the fall, winter, and spring, and have brought out the occurrence of some important differences in the variation of cold hardiness of larvae from ponderosa, limber, and lodgepole pine. Chart II shows the difference in cold hardiness of the larvae from the three hosts, and Table I the critical ranges of the larvae at different times of the season.

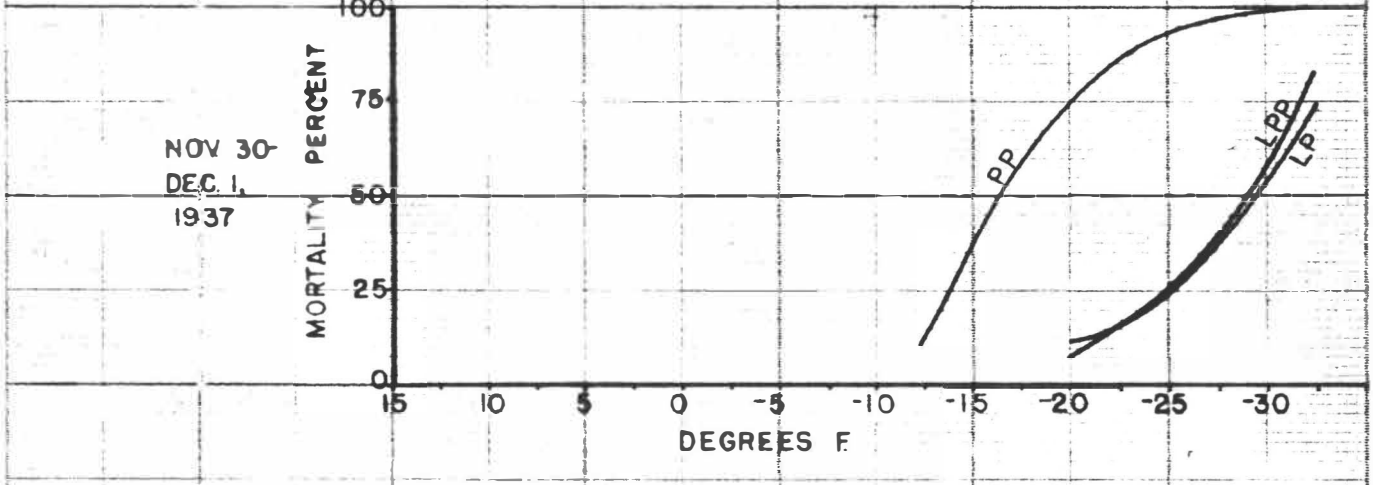
When the tests were made on Nov. 4-6 the hardiness of ponderosa and limber pine larvae was nearly the same. Lodgepole pine larvae were approximately 5 to 10 degrees more cold hardy. The variation of the hardiness of lodgepole pine larvae may be due to the conditioning that they received before the infested logs were collected and stored at Redfeather Lakes. The lodgepole was collected at a higher altitude and farther north where the October temperatures were colder. The bark on lodgepole pine is very thin and it is possible that conditioning of the larvae does not lag as much as it does with the larvae in the thicker bark species. This evidence of the effect of bark thickness is substantiated by the fact that the lodgepole pine larvae lose their cold hardiness in the spring with the approach of warm weather more rapidly than the larvae in the other two hosts.

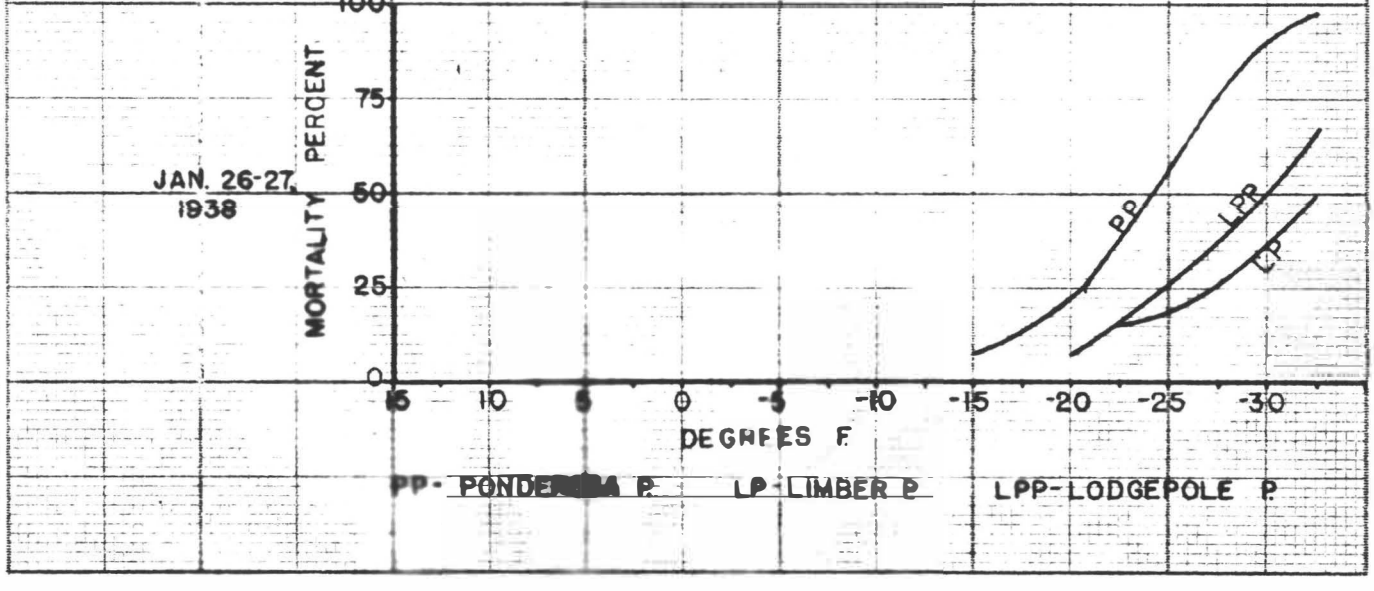
From Nov. 4 to Nov. 30 the cold resistance of the limber pine larvae increased approximately 25 degrees, lodgepole pine larvae approximately

CHART II

CRITICAL TEMPERATURES IN RELATION TO HOST



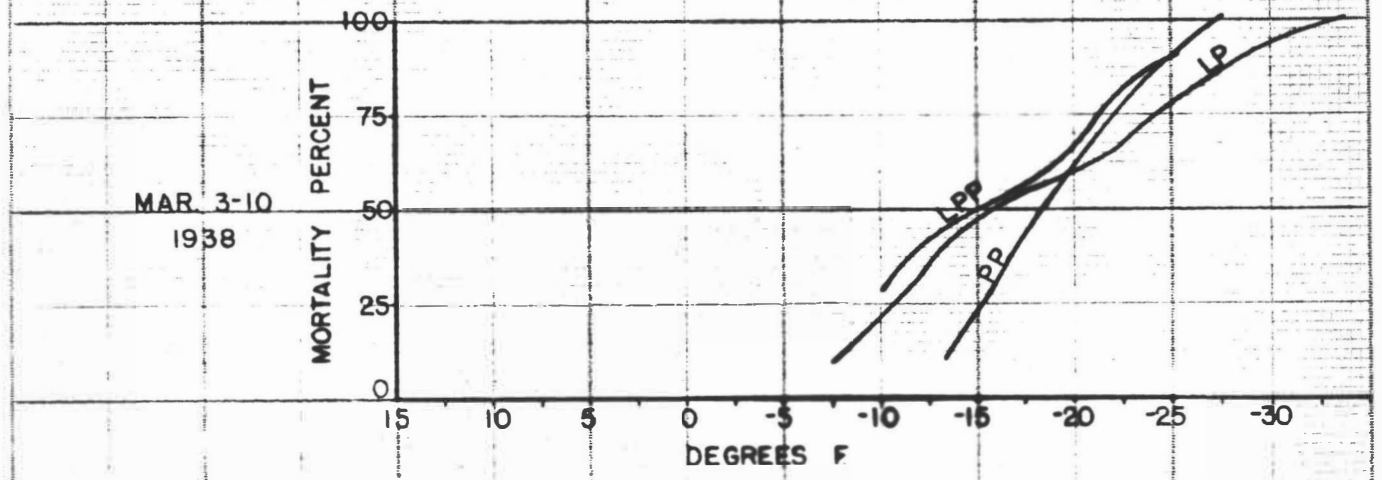


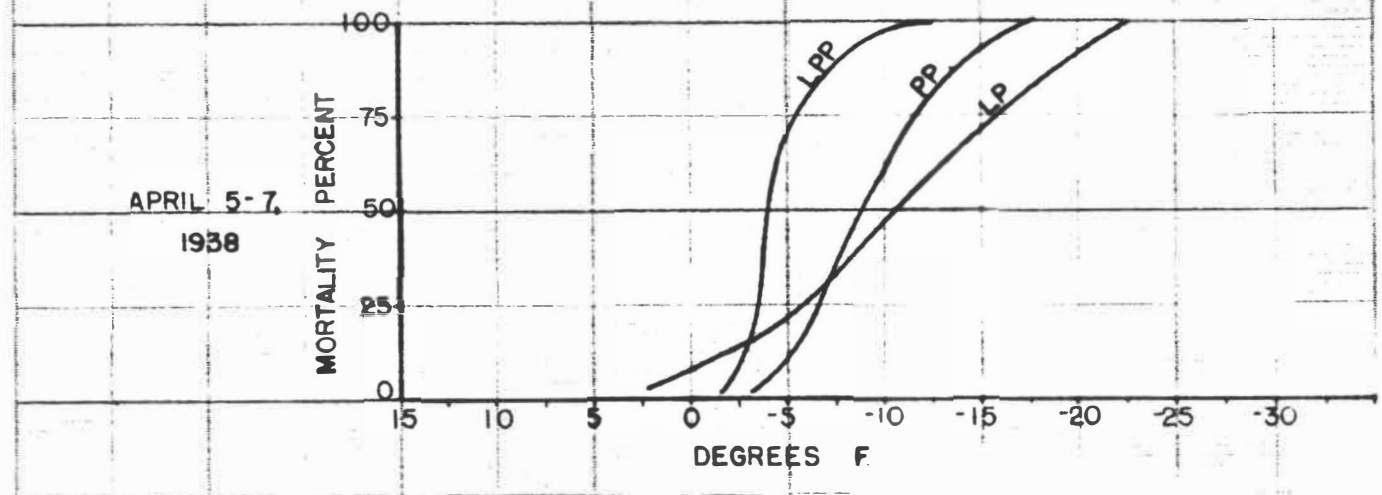


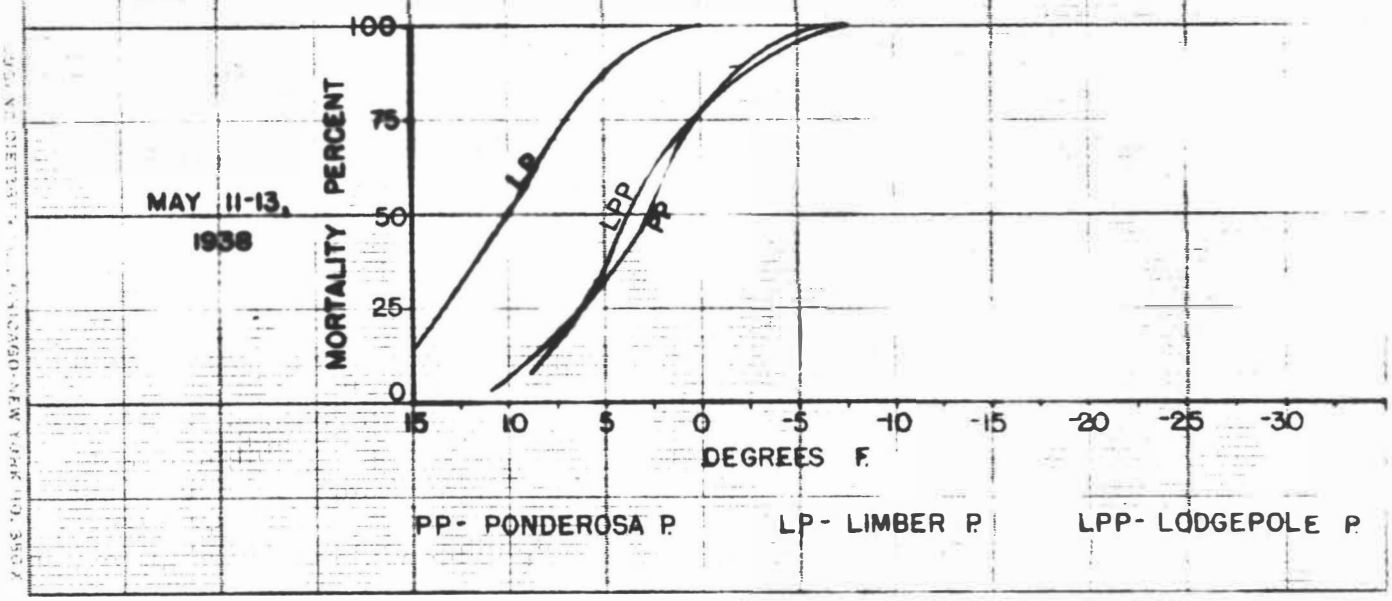
PP- PONDEROSA P. LP-LIMBER P. LPP-LODGEPOLE P.

CHART II CONT.

CRITICAL TEMPERATURES IN RELATION TO HOST







PP- PONDEROSA P. LP- LIMBER P. LPP- LODGEPOLE P.

20 degrees, and ponderosa pine larvae approximately 15 degrees. The cold hardiness of limber and lodgepole pine larvae was nearly the same on Nov. 30 and about 10 degrees more hardy than ponderosa pine larvae.

The limber and lodgepole larvae had nearly reached their maximum cold hardiness by Nov. 30 whereas the hardiness of the ponderosa pine larvae increased after Nov. 30.

The tests on Jan. 26-27 were believed to have been made at a time when the larvae had reached their maximum hardiness. The lowest temperature obtainable with the low temperature cabinet was -32½ degrees and not low enough to determine the ultimate critical point. Judging from the difference in the cold hardiness of the larvae over the first part of the critical range the ponderosa pine larvae were 7½ degrees less hardy than limber pine and 5 degrees less than lodgepole pine.

The critical ranges of the larvae from the three hosts in tests of March 3-10 were very close together except that there was a small percent of the limber pine larvae which were more hardy, making the initial and ultimate critical points unusually far apart.

In the April 5-7 tests there was a more pronounced difference in the hardiness of the larvae from the three hosts. The lodgepole pine larvae were the least hardy, being about 5 degrees less hardy than ponderosa pine larvae, and 7½ degrees less than the limber pine larvae.

On May 11-13 the mortality curves for the ponderosa and lodgepole pine larvae were nearly the same. The limber pine larvae were approximately 7½ degrees less hardy than the other larvae.

The fact that the larvae from limber pine are the most hardy of the larvae from the three hosts during the winter and least hardy during midspring after activity has started is very interesting. No explanation of this condition can be given at this time.

Critical Temperatures in Relation to Locality

During January and February cold resistance tests were made on larvae from ponderosa pine from four distant localities to determine whether or not there might be a difference in cold hardiness due to locality or biological strains. The infested logs were collected in the field in 40-inch lengths, brought to Fort Collins by truck, and the larvae removed and tested as soon as possible after arrival.

The localities from which material was collected and the date tested were Pine Lake in southern Utah on the Powell National Forest near Panguitch, Jan. 26-27; South Fork in southern Colorado on the Rio Grande National Forest, Feb. 9-10; Black Hills near Custer, South Dakota, on the Harney National Forest, Feb. 25-26; and Redfeather Lakes in northern Colorado on the Roosevelt National Forest, Jan. 26-27 and Feb. 9-10.

The mortality curves from the four localities are plotted on Chart III. The differences in hardiness at the four localities were not significant except that the Rio Grande N.F. larvae from southern Colorado were a little less hardy than the others.

Surplus material from the localities was stored at Redfeather Lakes with the infested logs collected in the fall. Tests were made with larvae

CHART III

CRITICAL TEMPERATURES IN RELATION TO LOCALITY

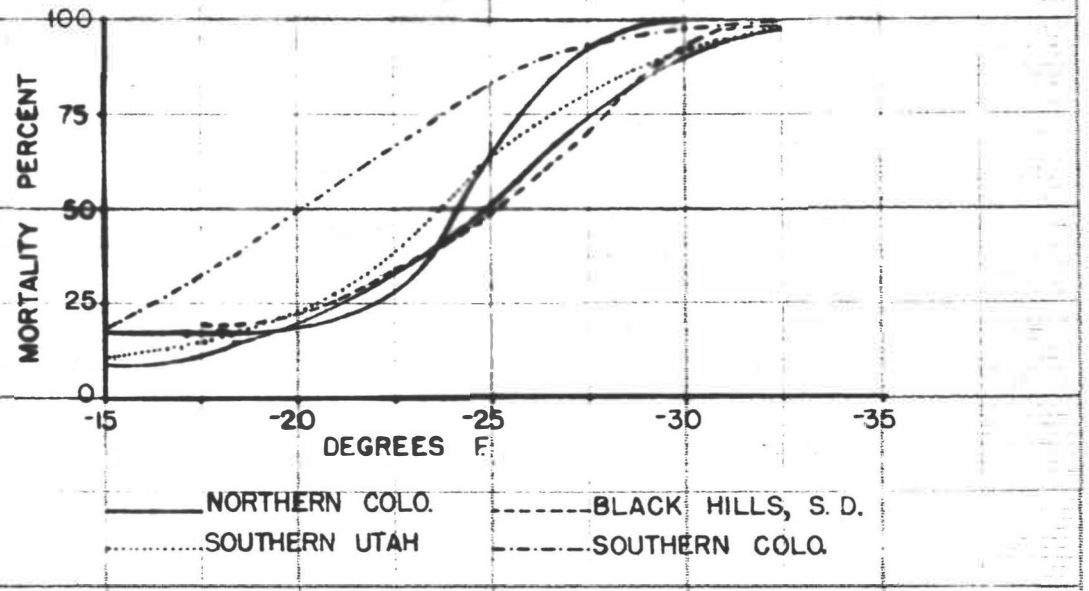
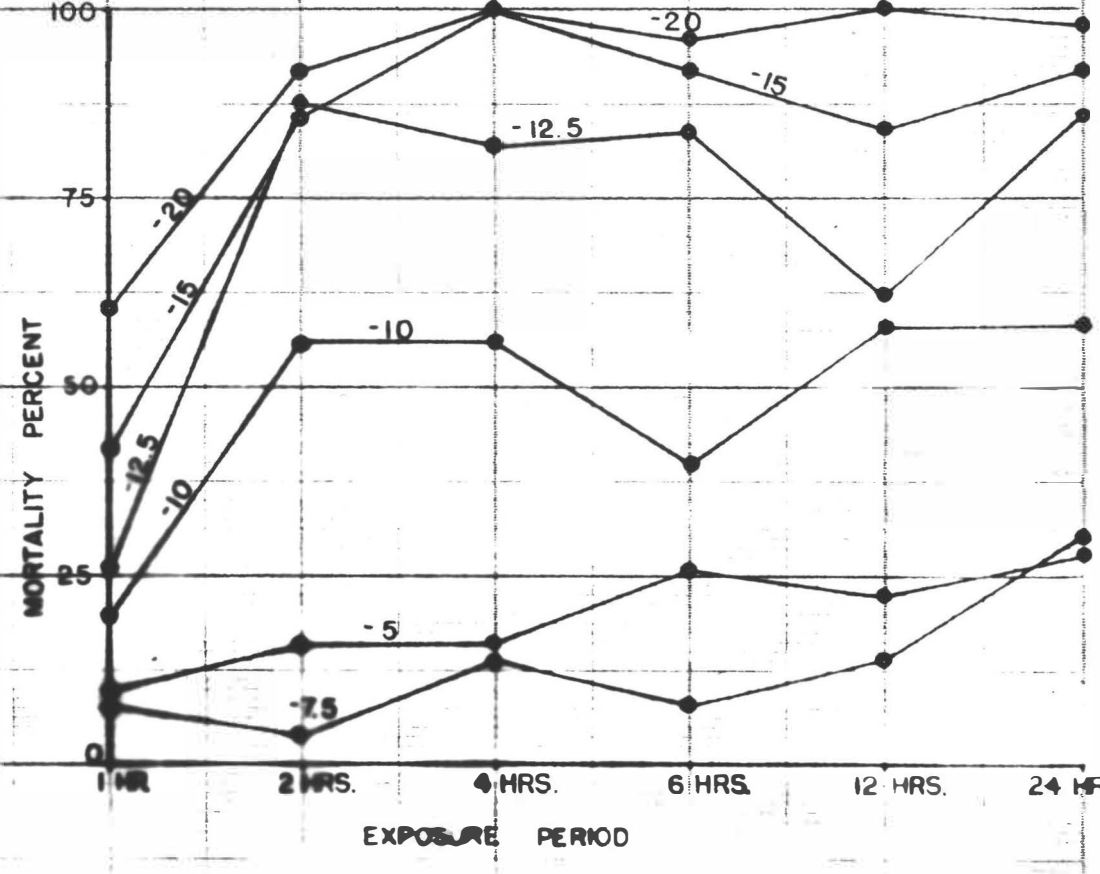


CHART IV

RELATION OF MORTALITY TO PERIOD OF EXPOSURE



from the Powell and Rio Grande National Forests along with the regular test on May 11-13. The data from this test are in the appendix under test No. 20. There was no significant difference in the hardiness from the three localities at that time.

The fact that the hardiness of the larvae from the four localities was nearly the same during midwinter does not necessarily mean that their hardiness during the spring and fall would be the same. Hardiness during spring and fall when their cold resistance is changing would probably vary in proportion to the coldness of the local weather. The tests eliminate the possibility of the existence of an inherited resistance at the rather distant localities in its natural distribution.

Relation of Mortality to Period of Exposure

Test No. 18 in the appendix was made to determine the effects of cold over periods longer than the 2 hours and 15 minutes used in all the other tests. Six petri dishes containing 50 larvae each were placed in the low temperature cabinet at the same time and the cabinet held at a uniform temperature. Tests were made at each $2\frac{1}{2}^{\circ}$ interval over the critical range. One dish of larvae was taken out at the end of the following periods: 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, and 24 hours. The results of this test are presented in Chart IV. There was a large difference in the mortality between larvae exposed 1 hour and 2 hours, but there appeared to be no significant difference in the mortality of those exposed for a period of 2 hours up to 24 hours.

The mortality curves run up slightly with longer periods of exposure at temperatures near the initial critical point, but this may not be sufficient to be significant. The results show that death or injury that later ends in death occurs within 2 hours and that exposures up to 24 hours does not cause a significantly greater mortality.

Experiment to Determine Effect of Warm Temperatures after Exposure

In order to determine whether there might be additional mortality of the larvae due to the shock of being suddenly taken from extremely low temperatures and exposed to room temperature, another experiment was incorporated in test No. 13, see appendix. At each exposure two dishes of larvae, 50 in each, from ponderosa pine, were placed in the cabinet instead of one as usual. After exposure one of the dishes was immediately placed at 32° F. and left at that temperature for 2 hours and 15 minutes before being placed at room temperature. The other dish was left at room temperature after being taken from the cabinet.

There was no significant difference in the mortality between the two methods of handling the larvae and therefore it was not deemed necessary to allow the larvae to warm up gradually after exposure.

SUMMARY OF WEATHER RECORDS

A recording thermograph and Weather Bureau type recording maximum and minimum thermometers were installed in the storage shelter at

Redfeather Lakes, Colo., on Nov. 8, 1937. The chart on the thermograph was changed and the recording thermometer read and reset every Monday morning by one of the enrollees at the Redfeather Lakes CCC Camp. The clock in the thermograph stopped on several occasions leaving some blanks in the records.

A table in the appendix (page 27) contains the daily maximum and minimum temperatures taken from the thermograph charts from Nov. 9 to May 22. The temperatures are summarized in the following table:

Summary of Temperatures at Redfeather Lakes, Colo., Nov. 9, 1937 to May 22, 1938, Inclusive.

Month	Maximum Temperature		Minimum Temperature	
	Degrees F.	Date	Degrees F.	Date
November	55	9 & 11	10	30
December	47	29	- 4	Between 8 - 12
January	43	14 & 15	1	25
February	43	11, 26 & 27	- 3	Between 16 - 20
March	52	20	7	27 & 30
April	64	25	2	1,2,6 & 7
May	59	13 & 14	13	7

The minimum for the period was -4° which occurred between December 8 to 12. The winter was a mild one with no extremely cold periods. At no time did the weather become cold enough to approach the critical range of the Black Hills beetle.

SUMMARY

The study of the low temperatures critical to the Black Hills beetle was carried out in an automatically controlled temperature cabinet. The larvae were exposed in samples of 50 in petri dishes. Each dish contained 50 cells or pits in paraffin wax in each of which a single larva was placed. The larvae were exposed for 2 hours and 15 minutes and then removed to room temperature. The exposures were made at 2-1/2 degree intervals over the critical range.

Between about Nov. 15 and April 15 when the larvae are extremely dormant it is necessary to place the larvae in individual 9x36 mm. vials with fresh pine phloem and stoppered with cotton after exposure in the cabinet. During this period a number of the larvae affected by the cold may not be killed outright by the cold but may live several weeks. These affected larvae are unable to feed and eventually die. The unaffected larvae start feeding on the phloem in about 3 days after exposure and develop into adults on the one vial of phloem. Some of the larvae are killed immediately by the exposure and turn black in 2 days.

Three species of pine infested with the Black Hills beetle were collected during the latter part of Oct. 1937 and stored in a shelter at Redfeather Lakes, Colo. This central storage facilitates collecting of the larvae for the tests and also provides identical conditioning temperatures for the larvae in the three hosts so that the difference in hardiness during the winter can be attributed to host differences and not to locality differences.

The relation of cold hardiness to time of year and to host is summarized in the following table:

Date	Critical Range of Black Hills Beetle Larvae in Three Hosts					
	Ponderosa Pine		Limber Pine		Lodgepole Pine	
	Initial	Ultimate	Initial	Ultimate	Initial	Ultimate
Nov. 4-6, 1937	2½	-12½	2½	-10	- 2½	-20
Nov. 30 - Dec. 1	-12½	-32½	-22½	68% at -32½	-22½	82% at -32½
Jan. 26-27, 1938	-20	98% at -32½*	-25	32% at -32½	-22½	60% at -32½
Mar. 3-10	-15	-27½	-10	-35	- 7½	90% at -25
Apr. 5-7	- 5	-17½	- 2½	-22½	- 2½	-12½
May 11-13, 1938	7½	-7½	15	0	7½	- 7½

*Percent mortality at the lowest temperature obtainable with the low temperature cabinet.

The larvae increase in hardiness with the advent of lower temperatures during the fall and winter. This hardiness is gradually lost as warmer weather arrives in the spring.

The lodgepole pine larvae were the most hardy of the larvae from ponderosa pine, limber pine, and lodgepole pine in early November; limber pine larvae were the most hardy of the three during the winter; and ponderosa pine larvae the most hardy of the three in the spring after larval activity had started. Limber pine larvae which were the most hardy during midwinter were the least hardy of the larvae from the three hosts during late spring after larval feeding had started.

During January and February infested ponderosa pine was brought by truck from southern Utah, southern Colorado, northern Colorado, and the Black Hills of South Dakota, and tested for cold hardiness. The differences in cold hardiness at the four localities were not significant except that the larvae from southern Colorado were a little less hardy than the others. Their hardiness during spring and fall would probably vary in proportion to the coldness of the local weather.

The results of tests of exposure from 1 hour to 24 hours show that death or injury that later ends in death occurs within 2 hours and that exposure up to 24 hours does not cause a significantly greater mortality.

The removal of the exposed larvae to room temperature as compared to gradual warming to room temperature produced no additional mortality.

Weather records kept at the storage shelter at Redfeather Lakes showed no low temperatures that approached the critical range of the larvae.

APPENDIX

Test No. 11. Black Hills Beetle Larvae. Nov. 4-6, 1937.

Material and Procedure:

Logs infested by Black Hills beetle larvae brought to Fort Collins from shelter at Redfeather Lakes, Colo., on Nov. 2. Larvae removed in warm room on Nov. 3 and stored immediately after removal at approximately 35° F. until used. Exposures were made Nov. 4-6 for 2 hours and 15 minutes in petri dishes. Samples of 50 larvae were used in each test except checks with 100 larvae.

Results:

Temperature Deg. F.	Percent Mortality by Hosts		
	Ponderosa Pine	Limber Pine	Lodgepole Pine
2½	6	10	4
0	26	50	10
- 2½	22	54	18
- 5	86	56	50
- 7½	96	96	78
-10	98	100	72
-12½	100	100	92
-15	--	--	90
-17½	--	--	96
Check	1	7	3

Test No. 12. Black Hills Beetle Larvae. Nov. 30 - Dec. 1, 1937.

Material and Procedure:

Infested logs brought to Fort Collins from Redfeather Lakes storage on Nov. 27. Larvae removed from bark on Nov. 29 in warm room and stored over night in refrigerator at 35° F. The larvae were stored during the day on ice. Exposures were made in petri dishes for 2 hours and 15 minutes on Nov. 30 and Dec. 1. Larvae put into vials with fresh phloem within 8 hours after exposure. Samples of 50 larvae used in each test.

Results:

Temperature Deg. F.	Percent Mortality by Hosts		
	Ponderosa Pine	Limber Pine	Lodgepole Pine
-10	20	--	--
-12½	16	--	--
-15	52	6	18
-17½	58	16	10
-20	74	12	8
-22½	86	18	20
-25	94	20	30
-27½	96	38	30
-30	98	60	56
-32½	100	68	82
Check	6	2	10

Test No. 13. Black Hills Beetle Larvae. Jan. 26-27, 1938.

Material and Procedure:

Infested ponderosa, limber, and lodgepole pine brought to Fort Collins on Jan. 24 from Redfeather. Infested ponderosa pine from Pine Lake, Powell National Forest, Utah, already in Fort Collins. Larvae removed from bark on Jan. 25 in warm room and stored immediately in refrigerator at approximately 35° F. until used. Exposures were made in petri dishes for 2 hours and 15 minutes on Jan. 26-27. Larvae put in vials with fresh pine phloem after exposure. Samples of 50 larvae used in tests with larvae from ponderosa, limber, and lodgepole pine from Redfeather storage, except where noted, and 100 larvae from ponderosa pine from Powell N. F., Utah.

Results:

Temp. Deg. F.	Percent Mortality by Hosts: Ponderosa P. A [1]	Ponderosa P. B [2]	Av. of A & B	Limber Pine	Lodgepole Pine	Ponderosa P. from Powell N.F.
-15	8	--	8	--	--	9
-17½	14	--	14	--	--	17
-20	22	16	19	22	12	20
-22½	32	56	44	22	10	34
-25	34	52	43	22	36	72
-27½	82	70	76	32	40 [3]	81
-30	91 [4]	90	90.5	46	42	92
-32½	100	94	97	32	60	100
Check	16	--	16	12	16	11

1. "A" larvae placed at 32° F. for 2 hrs. & 15 min. after removal from refrigerator.
2. "B" larvae placed at room temperature immediately after removal from " .
3. Sample of 47 larvae.
4. Sample of 48 larvae.

Test No. 14. Black Hills Beetle Larvae. Feb. 9-10, 1938.

Material and Procedure:

Infested ponderosa pine brought to Fort Collins on Feb. 5 from South Fork, Rio Grande National Forest, Colorado. Infested ponderosa pine brought to Fort Collins from Redfeather on Feb. 7. Larvae removed from bark in warm room on Feb. 8 and stored at 35° until used. Exposures were made in petri dishes for 2 hours and 15 minutes on Feb. 9-10. Larvae put in vials with phloem after exposure. Samples of 100 each were used in tests with larvae from the Rio Grande N. F., samples of 50 each with those from Redfeather storage.

Results:

Temperature Deg. F.	Percent Mortality	
	Ponderosa Pine from Redfeather	Ponderosa Pine from Rio Grande N. F.
-15	16	18
-17½	14	32
-20	16	51
-22½	26	61
-25	64	91
-27½	98	98
-30	100	94
-32½	100	100
Check	6	13

Test No. 15. Black Hills Beetle Larvae. Feb. 25-26, 1938.

Material and Procedure:

Infested ponderosa pine brought to Fort Collins from Custer, South Dakota, on Feb. 24. Larvae removed from bark on Feb. 25 and stored at 35° until used. Exposures were in petri dishes for 2 hours and 15 minutes on Feb. 25-26. Larvae put in viels with phloem after exposure. Samples of 100 larvae used in all tests.

Results:

Temperature Deg. F.	Percent Mortality Ponderosa Pine from Custer, S. D.
-17½	24
-20	22
-22½	38
-25	51
-27½	65
-30	91
-32½	98
Check	23

Test No. 16. Black Hills Beetle Larvae. March 3-5, 1938.

Material and Procedure:

Infested ponderosa, limber, and lodgepole pine brought down from Redfeather on March 2, larvae removed same day and stored on ice until used. Exposures made in petri dishes for 2 hours and 15 min. on March 3-5. Larvae put into vials with fresh pine phloem after exposure. Samples of 50 larvae were used in all tests.

Temperatures below $-32\frac{1}{2}^{\circ}$ obtained with dry ice placed in cabinet. Petri dishes sealed with Scotch tape to keep out CO_2.

Results with lodgepole pine larvae discarded because the larvae were very tiny which resulted in erratic results. The tiny larvae are more delicate and are not adapted to the present method of testing cold hardiness.

Results:

Temperature Deg. F.	Percent Mortality by Hosts	
	Ponderosa Pine	Limber Pine
-15	20	--
$-17\frac{1}{2}$	40	--
-20	64	--
$-22\frac{1}{2}$	82	62
-25	86	62
$-27\frac{1}{2}$	100	78
-30	100	98
$-32\frac{1}{2}$	100	98
-35	--	100
$-37\frac{1}{2}$	--	100
-40	--	100
Check	20	--

Test No. 17. Black Hills Beetle Larvae. March 8-10, 1938.

Material and Procedure:

Infested ponderosa pine brought to Fort Collins from Redfeather on March 7. Larvae removed from bark on March 8 and held on ice until used. Exposures made in petri dishes for 2 hours and 15 minutes on March 8-10. Larvae put into vials with fresh pine phloem after exposure. Samples of 50 larvae used in all tests.

Test No. 17 completes Test No. 16 for limber and lodgepole pine.

Results:

Temperature Deg. F.	Percent Mortality by Hosts	
	Limber Pine	Lodgepole Pine
-10	20	28
-12½	32	46
-15	54	52
-17½	56	54
-20	58	60
-22½	62	86
-25	92	90
Check	10	8

Test No. 18. Black Hills Beetle Larvae. March 15-22, 1938.

Material and Procedure:

Infested ponderosa pine brought to Fort Collins from Redfeather on March 14. Larvae removed from bark on March 15 for tests that day. Larvae removed from bark on March 16 for tests that day. The weather was becoming warm on March 17 and sufficient larvae were removed for succeeding days and stored at 35° in refrigerator until used. Exposures made in petri dishes. Larvae put into vials with fresh pine phloem after exposure. Samples of 50 larvae were used in all tests.

Results:

Temp. Deg. F.	Exposure Time and Percent Mortality						
	Check	1 Hour	2 Hours	4 Hours	6 Hours	12 Hours	24 Hours
- 5	12	10	16	16	26	22	28
- 7½	--	8	4	14	8	14	30
-10	--	20	56	56	40	58	58
-12½	--	26	88	82	84	62	86
-15	6	42	86	100	92	84	92
-20	--	60	92	100	96	100	98
-25	6	88	100	100	100	100	100

Test No. 19. Black Hills Beetle Larvae. April 5-7, 1938.

Material and Procedure:

Infested ponderosa, limber, and lodgepole pine brought to Fort Collins from Redfeather on April 4. Larvae removed from bark on April 5 and stored in refrigerator at approximately 35° until used. Exposures made in petri dishes for 2 hours and 15 minutes on April 5-7. The larvae were not put into vials with phloem because it was possible to differentiate the uninjured larvae from the injured and dead ones. Samples of 50 larvae were used in all tests.

Results:

Temperature Deg. F.	Percent Mortality by Hosts		
	Ponderosa Pine	Limber Pine	Lodgepole Pine
2½	--	2	0
0	8	16	0
- 2½	2	14	4
- 5	6	20	72
- 7½	38	48	96
-10	60	40	100
-12½	86	60	100
-15	92	82	100
-17½	100	78	98
-20	100	88	100
-22½	100	100	100
-25	100	100	100
-27½	100	100	100
Check	2	2	2

Test No. 20. Black Hills Beetle Larvae. May 11-13, 1938.

Material and Procedure:

Infested ponderosa, limber, and lodgepole pine brought to Fort Collins from Redfeather on May 10. Material from Rio Grande National Forest and Powell National Forest was collected during January and February and stored at Redfeather for the remainder of winter. Larvae removed from the bark on May 11 and stored at approximately 35° until used. Exposures were made in petri dishes for 2 hours and 15 minutes. Vials with phloem were not used. Samples of 50 larvae were used in all tests.

Results:

Temp. Deg. F.	Percent Mortality by Hosts: Ponderosa P. from Roosevelt N.F.	Ponderosa P. from Powell N. F.	Ponderosa P. from Rio Grande N.F.	Limber P.	Lodgepole P.
12½	4	2	4	32	4
10	20	0	4	48	8
7½	12	16	2	68	8
5	32	24	40	90	32
2½	46	26	74	98	66
0	92	80	94	100	86
- 2½	90	92	96	100	98
- 5	98	100	100	100	92
- 7½	100	100	100	100	100
-10	100	100	100	100	100
-12½	100	100	100	100	100

Summary of Daily Temperatures at Redfeather Lakes, Colorado, November 1937 - May 1938

Date	November		December		January		February		March		April		May	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1			43	23	38	19	36	28	41	27	17	2	44	33
2			41	22	36	23	30	22	44	25	30	2	50	34
3			38	26	26	9	38	24	45	29	35	18	40	28
4			26	16	35	12	29	20	33	19	42	24	36	25
5			30	18	--	12	25	13	27	15	42	26	35	23
6			--	24	21	12	33	12	31	15	26	2	30	17
7			20	6	27	7	42	6	36	17	20	2	35	13
8			--	--	28	7	42	28	34	11	34	10	39	22
9	55	30	--	--	23	14	34	24	38	22	51	26	46	23
10	54	29	--	-4*	23	16	40	31	32	23	43	26	53	26
11	55	28	--	--	30	7	43	28	41	26	--	--	53	35
12	34	25	--	--	--	--	38	17	48	32	--	--	47	31
13	36	24	23	14	30	22	32	12	42	29	--	--	59	33
14	47	28	27	14	43	30	34	10	32	29	--	--	59	30
15	35	25	36	18	43	27	20	0	32	25	--	--	48	36
16	39	26	35	21	34	25	--	--	45	22	--	--	56	34
17	34	20	30	20	34	23	--	--	47	20	--	--	54	33
18	26	12	24	12	26	17	--	-3*	37	16	48	29	57	34
19	32	19	21	5	27	11	--	--	40	25	51	31	52	30
20	42	24	25	0	22	7	--	--	52	28	52	28	44	29
21	36	29	33	18	35	10	28	12	45	12	56	30	37	30
22	40	21	29	11	40	23	27	15	22	9	36	29	44	31
23	47	25	26	15	28	8	25	10	38	18	40	30		
24	48	29	22	12	10	4	--	4*	44	21	45	33		
25	42	28	25	11	--	1*	--	--	37	20	64	40		
26	35	13	28	5	--	--	43	25	23	19	53	32		
27	26	14	39	23	41	31	43	24	33	7	43	28		
28	30	25	40	29	41	19	40	25	36	18	55	28		
29	27	15	47	25	32	10			21	11	58	31		
30	33	10	40	26	22	14			22	7	62	36		
31			35	22	37	21			23	12	44	33		

* Minimum temperature recorded by recording thermometer during the period that the thermograph was not running